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On the photochemical phosphorylation of imidazole

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SUMMARY

It has not proved possible to repeat the reported anaerobic photophosphorylation of imidazole to produce imidazole 1-phosphate. The involvement of an analogous reaction in mitochondrial oxidative phosphorylation is therefore further in doubt.

It has been claimed that imidazole is phosphorylated by inorganic phosphate in anaerobic aqueous solution, when irradiated with visible light in the presence of haematoporphyrin[1]. This reaction, as well as others previously described, form the basis for a model of mitochondrial oxidative phosphorylation involving a phosphorylated imidazolyl intermediate[1,2]. That such a reaction is possible is of course no evidence for or against the occurrence of an analogous reaction *in vivo*. Indeed Boyer[3] has presented the evidence against an intermediate in ATP formation in the actual enzymic systems.

In view of the interesting chemistry which would be involved, and to explore its generality, I have attempted to repeat the photophosphorylation of imidazole. I have been entirely unsuccessful in this attempt.

The experimental conditions were designed to reproduce those described by Tu and Wang[1]. Two solutions of pH 7.0 were prepared, Soln A contained haematoporphyrin ($2 \cdot 10^{-4}$ M), imidazole ($1 \cdot 10^{-3}$ M) and inorganic orthophosphate ($1 \cdot 10^{-1}$ M), and Soln B contained haematoporphyrin ($2 \cdot 10^{-4}$ M), imidazole ($1 \cdot 10^{-1}$ M) and inorganic orthophosphate ($1 \cdot 10^{-3}$ M). Carrier-free ^{32}P-labelled inorganic phosphate was added to each solution. The final specific radioactivity of the inorganic orthophosphate was 5 mCi/mmole for Soln A and 500 mCi/mmole for Soln B.

Three aliquots of each solution were placed into separate tubes. Two tubes were sealed with air in them, the control being covered with aluminium foil. The third tube was freed of air by freezing the contents, evacuating to a pressure of 0.1 Torr and after allowing the contents to thaw, introducing purified nitrogen (Air Products Research

Grade, < 1.0 ppm O_2) to a pressure of 800 Torr. This cycle was repeated 5 times, and the tube was finally sealed with a slight positive nitrogen pressure. The tubes were placed in a glass bath (containing water and ethylene glycol, 2:1, by vol.), and illuminated with a 500-W reflector photoflood lamp run at 75% of the rated voltage, with a yellow filter (Corning 3385). The bath temperature was maintained at 4 ± 1 °C by a copper coil through which was passed ethanol at -20 °C. Samples were illuminated until the porphyrin in the tubes containing oxygen appeared decolourised (approx. 36 h).

The samples were analysed by paper chromatography at 5 °C in 0.1 M aqueous potassium carbonate and ethanol (3.5:6.5, by vol.), the system of Rathlev and Rosenberg[4]. The paper was passed through a radiochromatogram scanner, and the compounds were located by spray reagent as described by Rathlev and Rosenberg[4]. Synthetic imidazole 1-phosphate, prepared by the method of Cramer *et al.*[5], was purified by preparative paper chromatography in the previous system, and was used as a marker.

When the samples were analysed, no trace whatsoever of radioactive imidazole 1-phosphate was found, although for Soln A less than 10%, and for Soln B less than 0.1% yield, based on imidazole, could have been detected. Synthetic imidazole 1-phosphate added before the start of an experiment was shown to survive the conditions. In the case of Soln A it was found to be essential to bring the pH to 10 before spotting on the paper chromatogram. Below this pH, different protonated forms of phosphate are present and give rise to two spots with streaking between them. In the case of Soln B there is a sufficient concentration of sodium carbonate in the chromatographic solvent and the problem does not arise. An attempt to repeat the colourimetric determination of imidazole and imidazole 1-phosphate as described by Tu and Wang[1], showed that it gave irreproducible results as ethanol, which acts as a reducing agent for diazonium cations, was present in the solution prior to the addition of diazotised sulphanilic acid.

The samples all appeared unaltered after irradiation, except for Soln B in the presence of light and air, where background counts were higher. If the inorganic orthophosphate in Soln B was replaced by adenosine 2′(3′)-monophosphate, a 10% yield of cyclic adenosine 2′:3′-monophosphate was obtained in the presence of light and air. There was no trace of adenosine 2′:3′-monophosphate production in the absence of oxygen. Adenosine 2′:3′-monophosphate was stable under the reaction conditions, and adenosine 2′(3′)-monophosphate was stable in the absence of imidazole.

I have failed to confirm the anaerobic photophosphorylation of imidazole by inorganic orthophosphate. The aerobic photophosphorylation of adenosine 2′(3′)-monophosphate in the presence of imidazole and haematoporphyrin resembles some earlier reported phosphorylation reactions[6]. I suggest that photosensitised oxidation of imidazole to a peroxide[7], and subsequent formation of a species which can act as a condensing agent may be involved in this reaction.

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